MAJOR ARTICLE

Emergence of Multidrug-Resistant *Salmonella enterica* Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States

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We describe a field investigation in New England that identified the emergence and epidemiology of new strains of multidrug-resistant *Salmonella,* Newport-MDRAmpC, and summarize the Center for Disease Control and Prevention's surveillance data for these infections. In Massachusetts, the prevalence of Newport-MDRAmpC among *Salmonella* serotype Newport isolates obtained from humans increased from 0% (0/14) in 1998 to 53% (32/60) in 2001 ($P < .001$). In a retrospective case-control study, infection with Newport-MDRAmpC was domestically acquired and was associated with exposure to a dairy farm. Isolates from both humans and cattle had indistinguishable or closely related antibiograms and pulsed-field gel electrophoresis patterns. Nationally, the prevalence of ceftriaxone-resistant *Salmonella* increased from 0.5% in 1998 to 2.4% in 2001; 85% of the isolates in 2001 were Newport-MDRAmpC, and at least 27 states have isolated these strains from humans, cattle, or ground beef. These data document the widespread emergence of Newport-MDRAmpC strains in the United States and show that the 5-fold increase in the prevalence of *Salmonella* resistant to expanded-spectrum cephalosporins, between 1998 and 2001, is primarily due to the emergence of Newport-MDRAmpC strains.

Each year in the United States, among humans, *Salmonella* strains cause an estimated 1.4 million infections, 16,000 hospitalizations, and nearly 600 deaths [1]. Approximately half of these infections occur in children [2]. Antimicrobial therapy is not essential for infections resulting in acute, self-limited gastroenteritis but can be life-saving for invasive infections [3]. Extended-spectrum cephalosporins (e.g., ceftriaxone) are commonly used, especially in children, for treatment of severe salmonellosis [3].

During the last 2 decades, antimicrobial-resistant *Salmonella* strains, which are associated with increased rates of hospitalization and greater morbidity and mortality, have emerged in many regions of the world, including the United States [4–7]. A multidrug-resistant (MDR) strain of *Salmonella* serotype Typhimurium definitive type 104 (DT104) (resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline) emerged across the United States during the 1990s [8, 9]. More recently, the emergence of resistance

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to extended-spectrum cephalosporins among nontyphoidal *Salmonella* isolates has been reported in the United States and has been attributed to plasmid-mediated resistance to AmpC (CMY-2) β-lactamase [10, 11]. The use of antimicrobial agents in livestock, including cattle, has been associated with the emergence of antimicrobial-resistant nontyphoidal *Salmonella* strains and with the dissemination and transmission of these strains to humans [8, 12, 13].

In 2000, the Centers for Disease Control and Prevention (CDC) and several state health departments identified a surge in the incidence of *Salmonella* serotype Newport, particularly MDR strains [14]. Like DT104, these strains (known as Newport-MDRAmpC) were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition, Newport-MDRAmpC isolates were resistant to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and exhibited decreased susceptibility to ceftriaxone (MIC, ⩾16 μg/mL). In November 2000, a veterinary diagnostic laboratory sent 2 *Salmonella* isolates from ill dairy cattle to the Massachusetts Department of Public Health (MDPH) for serotyping. These isolates had the Newport-MDRAmpC resistance pattern. Coincidentally, MDPH identified isolates from 2 ill children, 1 of whom attended day care on a dairy farm, as having the Newport-MDRAmpC resistance pattern. We conducted a field investigation that identified cattle on dairy farms as a reservoir for Newport-MDRAmpC. This report describes that investigation and reviews national surveillance data that document the rapid and widespread emergence of Newport-MDRAmpC in the United States.

PATIENTS, MATERIALS, AND METHODS

New England Investigation

Identification and laboratory testing of isolates. In Massachusetts, clinical laboratories routinely forward *Salmonella* isolates to MDPH for serotyping [15]; isolates obtained from animals are occasionally forwarded from veterinary diagnostic laboratories. In November 2000, after identification of the cluster of Newport-MDRAmpC isolates, MDPH posted an e-mail on the PulseNet system (described below) and contacted the veterinary diagnostic laboratories in New England and requested that they forward all recent *Salmonella* isolates from cattle to MDPH. In January and March 2001, during routine visits to farms and animal auction houses, the Massachusetts Department of Food and Agriculture, Bureau of Animal Health (MDFA), collected a convenience sample of stool from cows that had diarrhea.

Antimicrobial susceptibility testing was performed on all submitted *Salmonella* Newport isolates. MIC values were determined for the following 16 antimicrobial agents by use of the semiautomated Sensititre broth-microdilution system (Trek Diagnostics): amikacin, ampicillin, amoxicillin/clavulanic acid, apramycin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. Where established, NCCLS interpretive criteria were used, and American Type Culture Collection quality-control strains were used for all susceptibility testing, according to NCCLS guidelines [16]. Resistance to ceftiofur, an expanded-spectrum cephalosporin that is used only in veterinary medicine, was defined as an MIC of ⩾8 μg/mL [17]. All ceftiofur-resistant strains identified by Sensititre methods were tested for susceptibility to ceftriaxone by use of NCCLS-approved manual broth microdilution methods; decreased susceptibility to ceftriaxone was defined as an MIC of ⩾16 μg/mL (resistance was defined as an MIC of ⩾64 μg/mL) [18]. The term "MDRAmpC" refers to the antimicrobial resistance pattern that includes amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, and decreased susceptibility to ceftriaxone.

Salmonella Newport isolates were subtyped by pulsed-field gel electrophoresis (PFGE), by use of the restriction endonuclease *Xba*I (Roche), according to published methods [19], and pattern numbers were assigned according to CDC's national molecular subtyping network (PulseNet) protocol [20]. BioNumerics software (Applied Maths) was used to compare the PFGE patterns. *Salmonella* Newport isolates were tested by a colony polymerase chain reaction (PCR) to detect a 354-bp internal segment of the bla_{CMY} genes, by use of primer sequences provided by P. D. Fey (University of Nebraska Medical Center, Omaha).

Case-control study. In March 2001, to identify the epidemiologic and clinical characteristics of patients infected with Newport-MDRAmpC, we conducted a retrospective, case-control study of sporadic *Salmonella* Newport infections. A case patient was defined as a resident of Massachusetts or Rhode Island with a laboratory-confirmed Newport-MDRAmpC infection during the 2-year period from 1 April 1999 through 31 March 2001. Persons who had infections associated with a recognized outbreak or who were not residents of Massachusetts or Rhode Island were excluded. Case patients were compared with 2 control groups: (1) ill control subjects and (2) community control subjects. Ill control subjects were residents of Massachusetts or Rhode Island with a laboratory-confirmed, antimicrobial-susceptible *Salmonella* Newport infection during the same 2-year period used to define case patients. Using random-digit dialing methods, we matched case patients to community control subjects by use of age-frequency and telephone-prefix matching criteria. Community control subjects were excluded if they reported a history of diarrhea (⩾3 loose stools during a 24-h period) during the 30-day period before the interview. We sought to enroll up to 3 community control subjects/case patient. A questionnaire that addressed medical

history, use of antibiotics, contact with farm animals, day care, travel history, and food preferences was administered by telephone to case patients and control subjects. Case patients and ill control subjects were asked about exposures during the 7 days before onset of illness. Community control subjects were asked about exposures during the 7 days before their interview. Informed consent was obtained from all patients or their parents or guardians, and human-experimentation guidelines of the US Department of Health and Human Services and of the MDPH were followed in the conduct of this research.

We calculated odds ratios (ORs) and 95% confidence intervals (CIs), by use of (1) exact methods and (2) unconditional and conditional maximum likelihood estimation for unmatched and matched control subjects, respectively. Variables were considered for inclusion in a multivariable logistic-regression model if they had an observed significance level of <0.25 in an exact test of univariate association. Using these candidates, exact methods were used to fit a series of models by use of forward and backward selection principles. All statistical analyses were performed by use of SAS software (version 8; SAS Institute) and LogXact software (Version 4.1; Cytel Software).

Dairy farm investigations. In November 2000, after identification of the cluster of Newport-MDRAmpC isolates, MDPH contacted neighboring states and the veterinary diagnostic laboratories in New England and requested that they forward all recent *Salmonella* isolates from cattle to MDPH. Several cattle isolates from farms in Massachusetts and Vermont were identified as having the Newport-MDRAmpC resistance pattern and PFGE pattern. In March 2001, we were invited to visit a dairy farm in Massachusetts and one in Vermont, where Newport-MDRAmpC had been recently isolated from ill cows and persons who worked in these settings. Farm owners were asked about farm practices, including use of antimicrobials, calf milk replacer, feed, and movement of animals on to and off of the farm. We collected stool samples from cattle with a recent history of diarrheal illness and a convenience sample of stools from other cattle. We also collected samples from the bulk milk tank, haylage, grain feed, and silage. Isolation of *Salmonella,* serotyping, antimicrobial susceptibility testing, and PFGE were conducted at MDPH and VDH, as described above.

National Data

After completion of the New England field investigation, we reviewed CDC's national data, to determine the prevalence of Newport-MDRAmpC infections in the United States. State public health laboratories routinely receive *Salmonella* isolates from clinical laboratories as a part of public health surveillance [21]. State laboratories then electronically report serotyping results of laboratory-confirmed cases of *Salmonella* to CDC through the National *Salmonella* Surveillance System (NSSS). We reviewed data from NSSS and from 2 other national surveillance databases: the National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria and PulseNet. NARMS monitors the antimicrobial susceptibility patterns among *Salmonella* isolates from humans received at participating public health laboratories. During 2001, 17 public health laboratories in 15 states (CA, CO, CT, FL, GA, KS, MA, MD, MN, NJ, NY, OR, TN, WA, and WV) participated in NARMS, representing ~108 million persons (40% of the US population) under surveillance. During the period studied (1996–2001), participating NARMS sites, including MDPH, serotyped and forwarded every tenth non–Typhi *Salmonella* isolate received to CDC for susceptibility testing, as described elsewhere [14]. PulseNet, the national molecular subtyping network for foodborne pathogens, facilitates outbreak detection and investigation [22]. State and local public health laboratories participating in PulseNet, including the MDPH, subtype *Salmonella* isolates by PFGE using a standard protocol [19, 20] and submit PFGE patterns electronically to the national database at CDC. PFGE testing for *Salmonella* Newport was initiated in 1999. State and local public health laboratories in 45 states (all states except AL, MS, ND, MT, and NV) and 3 federal agencies (CDC, United States Department of Agriculture [USDA], and United States Food and Drug Administration [FDA]) were part of PulseNet during 2001. Although submitted PFGE patterns are predominantly from isolates obtained from humans, patterns are also submitted from isolates derived from animals and food. Submissions are not independent of each other; an increase in submissions usually occurs after an outbreak has been identified and communicated.

RESULTS

New England Investigation

Laboratory characterization of isolates. From July 1998 through December 2001, MDPH serotyped 4485 *Salmonella* isolates from humans; 211 (4.7%) were serotype Newport. Of these 211 isolates, 35 were received during the last 6 months of 1998, 46 were received during 1999, 70 were received during 2000, and 60 were received during 2001. Further characterization of 190 *Salmonella* Newport isolates identified 76 (40%) as being Newport-MDRAmpC. The prevalence of Newport-MDRAmpC among *Salmonella* Newport isolates from humans increased from 0% (0/14) in 1998 to 53% (32/60) in 2001 ($P < .001$, t test for trend) (figure 1*A*). Of the 76 Newport-MDRAmpC isolates, 43 (56%) met the criteria for resistance to ceftriaxone (MIC, $\geq$64 μg/mL), 18 (24%) met the criteria for resistance to kanamycin, 4 (5%) met the criteria for resistance to trimethoprim-sulfamethoxazole, and 1 (1%) met the criteria for resistance to gentamicin. Of the other 114 *Salmonella* Newport isolates from humans, 1 isolate was resistant to ceftriaxone and all but 2 of the antimicrobials defining the Newport-

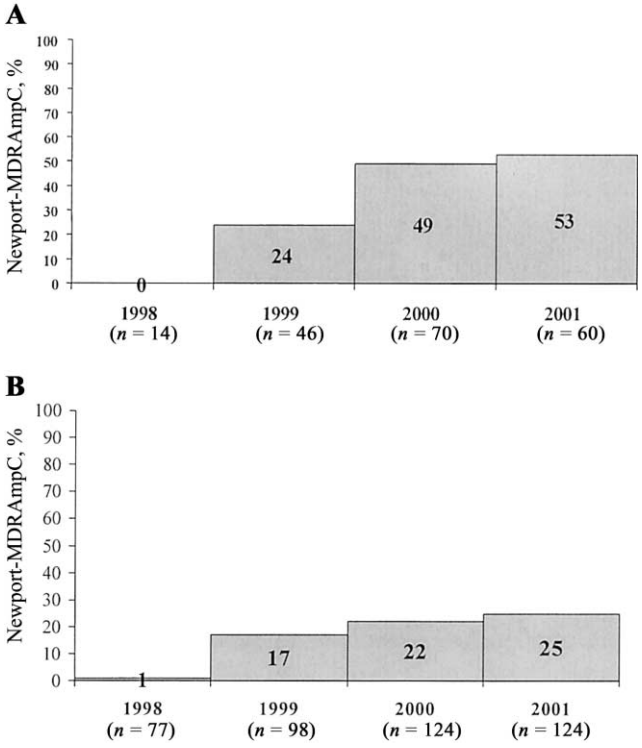

Figure 1. Percentage of *Salmonella* Newport isolates from humans that were Newport-MDRAmpC, 1998–2001.

MDRAmpC resistance pattern, including ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, ceftiofur, streptomycin, sulfamethoxazole, but not chloramphenicol or tetracycline. Two isolates were resistant to ampicillin, cephalothin, sulfamethoxazole, and tetracycline; 1 isolate was resistant to nalidixic acid and streptomycin; and 3 isolates were resistant to sulfamethoxazole. The remaining 107 *Salmonella* Newport isolates were pansusceptible. All the 190 *Salmonella* Newport isolates were susceptible to ciprofloxacin, apramycin, and amikacin.

Of the 76 Newport-MDRAmpC isolates, 10 PFGE patterns were identified, as illustrated in figure 2. The dendrogram generated from Dice coefficients of similarity indicates that all 10 PFGE patterns are highly related, with Dice values $\geqslant$85% and differing from each other by $\leqslant$3 bands. Among the Newport-MDRAmpC isolates, the most prevalent PFGE patterns were JJPX01.0014 (shared by 49 [64%] of the isolates) and JJPX01.0042 (shared by 12 [16%] of the isolates) (figure 2). The 1 MDR isolate that was resistant to ceftriaxone but did not meet the Newport-MDRAmpC criteria exhibited PFGE pattern JJPX01.0014. Of the remaining 113 *Salmonella* Newport isolates that did not meet the resistance pattern criteria for Newport-MDRAmpC, 62 PFGE patterns were identified, and these all differed by $\geqslant$3 bands from the 10 Newport-MDRAmpC PFGE patterns identified (data not shown).

Of the *Salmonella* isolates submitted to MDPH that were from animals, 59 were serotyped during 1999–2001; 19 were bovine isolates. Of the 19 isolates from cattle, 16 were received after MDPH contacted veterinary diagnostic laboratories serving the New England region in November 2000 and requested that they forward all recent *Salmonella* isolates from cattle to MDPH. Sixteen of the 19 isolates from cattle were *Salmonella* Newport; all were from dairy cattle. None of the other isolates from animals were *Salmonella* Newport. The 16 *Salmonella* Newport isolates were obtained from dairy cattle from 8 farms and 1 animal auction house, located in various regions of Massachusetts. Of the 16 *Salmonella* Newport isolates from dairy cattle, 15 were Newport-MDRAmpC, and 1 isolate was resistant to the same drugs as Newport-MDRAmpC and to kanamycin but not to chloramphenicol or tetracycline. Of the 15 Newport-MDRAmpC isolates, 10 (67%) were resistant to ceftriaxone, and 11 (69%) were resistant to kanamycin. PFGE testing of the isolates from cattle yielded 2 patterns, both of which were observed in isolates from humans; 11 (73%) were JJPX01.0014, and 4 (27%) were JJPX01.0181 (figure 2). All tested Newport-MDRAmpC isolates from humans ($n = 73$) and cattle ($n = 16$) were PCR-positive for a bla_{CMY} gene.

Case-control study. Of 46 the persons with laboratory-confirmed Newport-MDRAmpC infections who were eligible for inclusion in the case-control study, 34 (74%) were enrolled: 32 were residents of Massachusetts, and 2 were residents of Rhode Island. Of 68 persons with laboratory-confirmed, pansusceptible *Salmonella* Newport infections who were eligible to serve as ill control subjects, 37 (54%) were enrolled. The median age of enrolled case patients was 29 years (range, <1–83 years); 56% were female, and 88% were white/non-Hispanic (table 1). The most commonly reported symptoms among case patients were diarrhea (100%), abdominal cramps (77%), fever (77%), and vomiting (42%); 34% were hospitalized, and no deaths resulted. Three (9%) case patients reported having an underlying immunosuppressive condition: 2 had diabetes mellitus, and 1 was receiving chemotherapy for cancer. Case patients did not differ from ill control subjects by age, sex, or race/ethnicity. Case patients were more likely than ill control subjects to report having bloody diarrhea (52% vs. 18%; OR, 4.7 [95% CI, 1.4–17.9]) and to have visited, worked on, or lived on a dairy farm during the 7 days before onset of illness (21% vs. 3%; OR, 9.1 [95% CI, 1.1–432.0]). None of the case patients and 4 (11%) ill control subjects had traveled outside of the United States during the 7 days before onset of illness.

A total of 32 case patients were matched with 94 community control subjects. Case patients did not differ from matched community control subjects by sex or race/ethnicity (table 2). In matched univariate analysis, case patients were more likely than community control subjects to have visited, worked on,

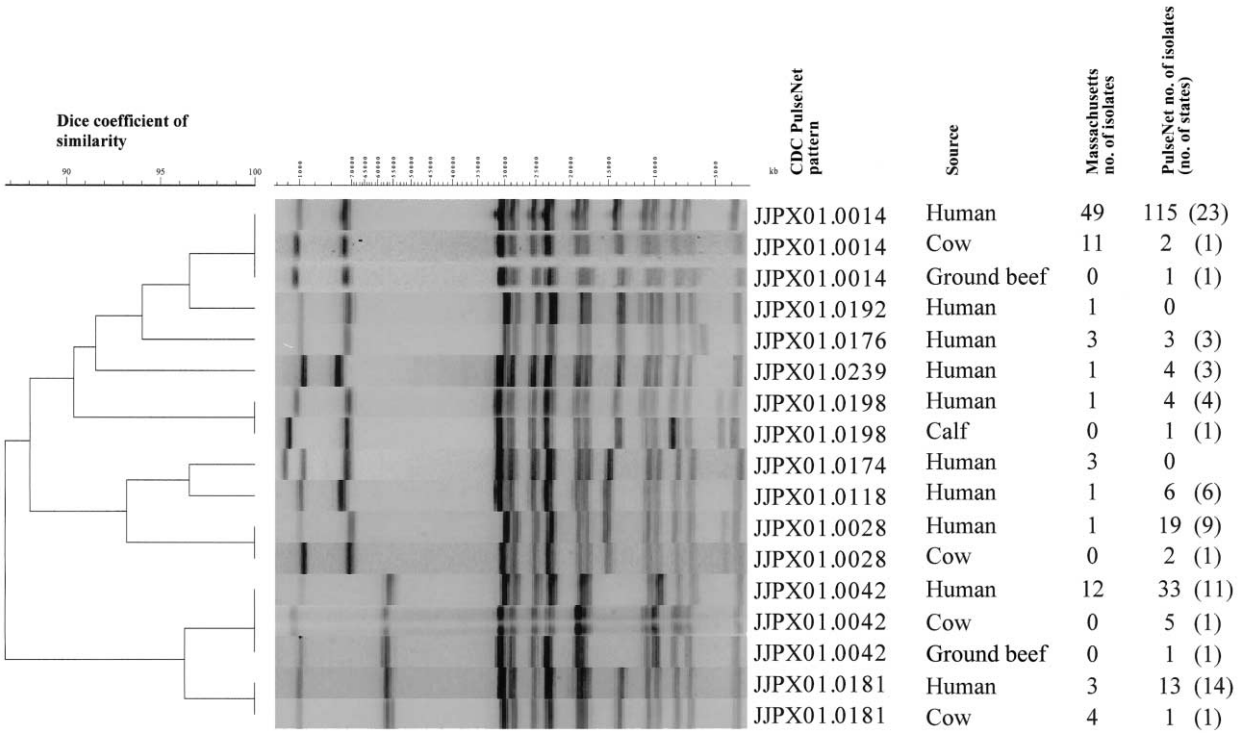

Figure 2. Newport-MDRAmpC pulsed-field gel electrophoresis (PFGE) patterns from Massachusetts and from the Center for Disease Control and Prevention's (CDC) national database, PulseNet, 1999–2001. Twenty-three states other than Massachusetts (CA, CO, CT, FL, GA, IA, KS, LA, MI, MN, MO, NM, NY, OH, OK, PA, RI, TN, TX, UT, VT, VA, and WI) reported PulseNet PFGE pattern JJX01.0014 for isolates obtained from humans, and 3 additional states (ME, NH, and SD) reported Newport-MDRAmpC–associated PFGE patterns that were not JJX01.0014.

or lived on a dairy farm during the 7 days before onset of illness (22% vs. 3%; matched OR, 16.7 [95% CI, 2.0–768.8]). Case patients were less likely than community control subjects to eat yogurt (39% vs. 73%; matched OR, 0.2 [95% CI 0.1–0.6]) during the 7 days before onset of illness. Consumption of beef during the 7-day exposure period was reported by 100% of case patients and by 91% of community control subjects, but this difference was not statistically significant (matched OR, 4.1 [95% CI, 0.6–∞]). No other exposures to food were found to be statistically significant (data not shown).

In multivariate analyses, case patients were more likely than matched community control subjects to have the following exposures during the 7 days before onset of illness/interview: visit, work on, or live on a dairy farm (adjusted OR, 12.2 [95% CI, 1.2-640]) and not consume yogurt (adjusted OR, 0.13 [95% CI, 0.03–0.4]).

Dairy farm investigations. We visited farm A in Massachusetts and farm B in Vermont, farms where Newport-MDRAmpC infections had been recently identified; these farms were both dairy farms and had 60 and 300 milking cows, respectively. The 2 owners of farm A were ill with fever and diarrhea in March 2000; 1 had a stool culture confirming Newport-MDRAmpC infection. The owners reported that several cattle were ill with diarrhea during 2000. MDFA visited farm A in January 2001 and isolated Newport-MDRAmpC from 2 calves ill with diarrhea. We visited farm A in March 2001, at which time 2 of 20 stool samples from asymptomatic cows yielded Newport-MDRAmpC. At farm B, the first case of a cow ill with diarrhea occurred in January 2001; during the ensuing 2 months, 12 cows became ill, and 6 died; stool samples from 2 ill cows yielded Newport-MDRAmpC. In February 2001, a farm worker on farm B became ill with fever and diarrhea, and laboratory testing of his stool sample yielded Newport-MDRAmpC. We visited farm B in March 2001, at which time 4 of 9 stool samples from 3 asymptomatic cows and from 1 calf that had died the morning of our visit yielded Newport-MDRAmpC. Although records were incomplete, ill cattle on farm A and farm B were treated with various antimicrobial agents; the use of ceftiofur and spectinomycin for the treatment of ill cattle with diarrhea was confirmed on farm B. Calves at both farm A and farm B were routinely fed calf milk replacer that contained antimicrobial agents, including tetracycline and neomycin. Samples of haylage, silage, wild-bird droppings, and the bulk milk tank were negative on both farms.

Table 1. Characteristics of and risk factors for infection with Newport-MDRAmpC, compared with those for ill control subjects (antimicrobial-susceptible *Salmonella* Newport), among residents of Massachusetts and Rhode Island, 1999–2001.

Variable	Newport-MDRAmpC case subjects (n = 34), no./total (%)	Ill control subjects (n = 37), no./total (%)	OR (CI)	P
Age, median (range), years	29 (0–83)	36 (0–81)		
Female	19/34 (56)	23/37 (62)	0.8 (0.3–2.2)	.77
White	30/34 (88)	32/37 (91)	0.7 (0.1–4.6)	.97
Rural/farm	5/34 (15)	0/37 (0)	8.2 (1.1–∞)	.04
Fever	23/30 (77)	18/32 (56)	2.6 (0.8–9.1)	.15
Vomiting	13/31 (42)	11/36 (31)	1.6 (0.5–5.1)	.48
Cramps	23/30 (77)	28/35 (80)	0.8 (0.2–3.2)	.98
Diarrhea	34/34 (100)	35/37 (95)	2.3 (0.2–∞)	.54
Bloody stool	16/31 (52)	6/33 (18)	4.7 (1.4–17.9)	.01
Antibiotic treatment for illness	20/32 (63)	26/35 (74)	0.6 (0.2–1.9)	.44
Antibiotic treatment before specimen collection	4/31 (13)	5/32 (16)	0.8 (0.1–4.2)	1.0
Hospitalized	11/32 (34)	7/36 (19)	2.2 (0.6–7.7)	.26
Treated with antacids during the 4 weeks before onset of illness	6/33 (18)	9/35 (26)	0.6 (0.2–2.4)	.65
International travel during the 7 days before onset of illness	0/34 (0)	4/37 (11)	0.2 (0–1.6)	.14
Any exposure to an animal pet	18/25 (72)	19/37 (51)	2.4 (0.73–8.53)	.17
Exposure to a dairy farm[a]	7/34 (21)	1/37 (3)	9.1 (1.1–432.0)	.04

NOTE. CI, confidence interval; OR, odds ratio.

[a] Visited, lived on, worked on, attended day care at, or received manure from a dairy farm.

Four months after our visit, we again interviewed the owners of farm A and those of farm B; all reported recent onset of diarrheal illness in their dairy cattle.

National Data

Although the number of laboratory-confirmed *Salmonella* infections reported to CDC via NSSS decreased from 36,995 cases in 1996 to 31,607 cases in 2001, the number of *Salmonella* isolates that were serotype Newport increased from 1985 (5%) in 1996 to 3149 (10%) in 2001. In 2001, Newport was the third-most common *Salmonella* serotype isolated, after Typhimurium and Enteriditis. During this 6-year period, 8046 serotyped nontyphoidal *Salmonella* isolates were analyzed by NARMS; 522 (7%) were serotype Newport. Of the *Salmonella* Newport isolates, 76 (15%) were Newport-MDRAmpC. The prevalence of Newport-MDRAmpC among *Salmonella* Newport isolates was 0% (0/51) in 1996 and 1% (1/77) in 1998 and increased to 25% (31/124) in 2001 ($P < .0001$, test for trend) (figure 1*B*). The prevalence of *Salmonella* with resistance to ceftriaxone was 0.5% (8/1465) in 1998 and increased to 2.4% (34/1419) in 2001. In 2001, 85% (29/34) of all ceftriaxone-resistant *Salmonella* isolates were Newport-MDRAmpC. By 2001, Newport-MDRAmpC was identified in all 17 participating NARMS sites, representing 15 states. The median age of patients with Newport-MDRAmpC infection was 33 years (range, <1–81 years); 37% of patients were <18 years of age, and 57% were female. Among the 76 Newport-MDRAmpC isolates, 63 (83%) met the NCCLS criteria for resistance to ceftriaxone (MIC, ⩾64 μg/mL). In addition, 13 (17%) were also resistant to kanamycin, 8 (11%) were also resistant to trimethoprim-sulfamethoxazole, and 5 (7%) were also resistant to gentamicin. All the Newport-MDRAmpC isolates were susceptible to nalidixic acid, ciprofloxacin, amikacin, and apramycin (table 3). Of the 76 Newport-MDRAmpC isolates, 1 was isolated from blood. Of the 446 *Salmonella* Newport isolates that were not Newport-MDRAmpC, 17 (4%) were resistant to ⩾2 antimicrobials, 22 (5%) were resistant to 1 antimicrobial, and 407 (91%) were pansusceptible.

During 1999–2001, a total of 2252 *Salmonella* Newport isolate PFGE patterns from 33 states (excluding MA) and 2 federal agencies (USDA and FDA) were analyzed by PulseNet. PFGE patterns from 212 (9%) of the isolates submitted by 26 states and USDA were indistinguishable from 1 of the 10 closely related Newport-MDRAmpC PFGE patterns submitted by MDPH (figure 2). Data on susceptibility to antimicrobials for these isolates were not uniformly submitted to CDC and, therefore, could not be analyzed. Of 212 isolates with Newport-

Table 2. Characteristics of and risk factors for infection with Newport-MDRAmpC, compared with those for matched community control subjects, among residents of Massachusetts and Rhode Island, 1999–2001.

Variable	Newport-MDRAmpC case subjects (n = 32), no./total (%)	Community control subjects (n = 94), no./total (%)	Matched OR (CI)	P
Age, median (range), years	29 (0–83)	31.5 (0–89)		
Sex, female	17/32 (53)	55/94 (59)	0.8 (0.4–1.9)	.76
White	28/32 (88)	87/94 (93)	0.5 (0.1–2.9)	.55
Rural/farm	5/32 (16)	11/94 (12)	1.5 (0.3–7.5)	.8
Antibiotics during 7 days before onset of illness/past week	4/31 (13)	3/93 (3)	4.8 (0.7–54.6)	.14
Any exposure to an animal pet	16/23 (70)	52/94 (55)	1.8 (0.58–6.17)	.38
Exposure to a dairy farm[a]	7/32 (22)	3/94 (3)	16.7 (2.0–768.8)	.003
Food consumed				
Milk	27/32 (84)	85/94 (90)	0.6 (0.2–2.3)	.50
Ice cream	22/32 (69)	81/94 (86)	0.3 (0.1–1.0)	.04
Yogurt	12/31 (39)	69/94 (73)	0.2 (0.1–0.6)	.002
Any beef	32/32 (100)	86/94 (92)	4.1 (0.6–∞)	.18

NOTE. CI, confidence interval; OR, odds ratio.

[a] Visited, lived on, worked on, attended day care at, or received manure from a dairy farm.

MDRAmpC PFGE patterns, 197 were obtained from humans, 11 were obtained from cattle, 2 were obtained from samples of ground beef, 1 was obtained from a horse, and 1 was obtained from a veterinary hospital environmental sample. Of the 197 isolates from humans that had Newport-MDRAmpC PFGE patterns, 115 (58%) were JJXP01.0014. These 115 strains had been identified in 23 states, including geographically distant states, such as California, Florida, Kansas, Michigan, and New York (figure 2). The isolates from cattle were submitted from Michigan, Minnesota, and Vermont; the isolates from ground beef were collected during 1999 as part of an investigation of an outbreak in Virginia and routine USDA sampling in Washington; and the equine and environmental isolates were collected as part of an outbreak in a veterinary hospital in Michigan during 2001.

DISCUSSION

Since 1998, Newport-MDRAmpC strains of *Salmonella* have rapidly emerged throughout the United States. These strains are resistant to 9 antimicrobials, have either decreased susceptibility to or resistance to extended-spectrum cephalosporins, such as ceftriaxone, and are sometimes resistant to trimethoprim-sulfamethoxazole. This resistance profile renders them resistant to most available antimicrobial agents approved for the treatment of salmonellosis, particularly in children. The emergence of Newport-MDRAmpC infections in humans has occurred in the United States despite an overall decrease in the incidence of *Salmonella* infections since 1996 [23]. In 1998, 0.5% of all *Salmonella* strains in NARMS were resistant to ceftriaxone [11], and these were predominantly *Salmonella* Typhimurium. By 2001, however, 2.4% of all *Salmonella* strains tested in NARMS were resistant to ceftriaxone; 85% of these were Newport-MDRAmpC strains. Thus, the increase in prevalence ceftriaxone-resistant *Salmonella* strains that occurred between 1998 and 2001 has been largely driven by the rapid emergence of Newport-MDRAmpC strains. We estimate that >2% of the estimated >1 million *Salmonella* infections in 2001 were caused by Newport-MDRAmpC strains.

The emergence of Newport-MDRAmpC strains in humans has coincided with the emergence of Newport-MDRAmpC infections in cattle. In Massachusetts and Vermont, we identified several *Salmonella* Newport isolates from cattle as Newport-MDRAmpC. Other studies have reported recent outbreaks of Newport-MDRAmpC among dairy cattle in several states, including California, Massachusetts, Maryland, New York, Pennsylvania, Vermont, and Wisconsin [24, 25] (CDC unpublished data). The prevalence of *Salmonella* Newport among dairy cattle may vary over time, on the basis of findings by different studies. In a cross-sectional study of California dairy farms in 1989, *Salmonella* was isolated in 12 (16%) of 75 randomly selected farms; *Salmonella* Newport was isolated in 6 (8%) [26]. In 1996, among dairy cows sampled at slaughter, *Salmonella* Newport was an uncommon serotype identified [27]. In more recent data (from the National Veterinary Services Laboratory [NVSL]), which are based on voluntary submissions of isolates from ill cattle by veterinarians and producers and are not based on any systematic sampling scheme, the proportion attributable

Table 3. Antimicrobial susceptibility of Newport-MDRAmpC, compared that of other *Salmonella* Newport isolates, in Massachusetts (1998–2001) and in National Antimicrobial Resistance Monitoring System (NARMS) (1996–2001), by use of 2002 NCCLS criteria.

		Massachusetts		NARMS	
Antimicrobial agent	Breakpoint for resistance, MIC μg/mL[a]	Newport-MDRAmpC (*n* = 76)	Other *Salmonella* Newport (*n* = 114)	Newport-MDRAmpC (*n* = 76)	Other *Salmonella* Newport (*n* = 446)
Ampicillin	⩾32	76 (100)	2 (2)	76 (100)	13 (3)
Chloramphenicol	⩾32	76 (100)	1 (1)	76 (100)	12 (3)
Streptomycin	⩾64	76 (100)	1 (1)	76 (100)	19 (4)
Sulfamethoxazole	⩾512	76 (100)	3 (4)	76 (100)	26 (6)
Tetracycline	⩾16	76 (100)	2 (2)	76 (100)	16 (4)
Cephalothin	⩾32	76 (100)	2 (2)	76 (100)	6 (1)
Amoxicillin-clavulanic acid	⩾32	76 (100)	0	76 (100)	3 (<1)
Cefoxitin[b]	⩾32	76 (100)	0	60 (100)	0
Ceftiofur	⩾8	76 (100)	0	76 (100)	6 (1)
Ceftriaxone[c]	⩾64	43 (56)	1 (0)	63 (83)	0
Kanamycin	⩾64	18 (24)	0	13 (17)	4 (<1)
Gentamicin	⩾16	1 (1)	0	5 (7)	8 (2)
Trimethoprim-sulfamethoxazole	⩾4/76	4 (5)	0	8 (11)	6 (1)
Nalidixic acid	⩾32	0	1 (1)	0	1 (<1)
Ciprofloxacin	⩾4	0	0	0	0
Amikacin	⩾64	0	0	0	0
Apramycin	⩾32	0	0	0	0

[a] MIC values listed are the 2002 NCCLS breakpoint criteria for resistance.
[b] In NARMS, cefoxitin was tested only in 2000 and 2001.
[c] Ceftriaxone susceptibilities on the basis of NCCLS-approved manual broth microdilution methods.

to serotype Newport increased from 4% in 1999 to 25% in 2001 (personal communication, K. Ferris [NVSL]). This increase appears to be largely driven by Newport-MDRAmpC strains. In the NARMS Veterinary Report of 2000, 185 *Salmonella* Newport isolates from cattle were tested, and >70% of these were resistant to the antimicrobials that define the Newport-MDRAmpC resistance pattern [28]. The recent recognition of and increase in MDR *Salmonella* Newport isolates prompted the USDA National Animal Health Monitoring System to release a fact sheet in September 2002 entitled "What Veterinarians and Producers Should Know about Multidrug-Resistant Salmonella Newport" [29].

Our retrospective case-control study of sporadic Newport-MDRAmpC infections in residents of New England has demonstrated that infections are domestically acquired and are associated with direct exposure to a dairy farm. Our finding that yogurt consumption is protective is intriguing, since some studies have suggested that bacteria found in yogurt may prevent salmonellosis [30, 31]. The leading risk factor for illness in humans, direct exposure to a dairy farm during the 7 days before onset of illness, accounted for only 21% of cases.

We hypothesize that many of our cases of infection are associated with the handling or consumption of contaminated foods, as has been demonstrated in other epidemiologic studies of salmonellosis [13]. Beef and milk from dairy cattle may be a substantial source of Newport-MDRAmpC infections for the following reasons. We isolated Newport-MDRAmpC strains from both ill and healthy cattle on dairy farms. Approximately 18% of the nation's ground beef originates from dairy cattle, and *Salmonella* is frequently isolated from culled dairy cows at slaughter [32, 33]. In the national database, we identified 2 isolates from ground beef with PFGE patterns that were indistinguishable from the 2 most common Newport-MDRAmpC PFGE patterns observed in isolates from humans. Samples of ground beef derived from USDA-inspected chilled carcasses have yielded Newport-MDRAmpC [34]. Multistate outbreaks of MDR *Salmonella* Newport caused by other, less-resistant strains during the 1970s and 1980s were associated with the consumption of ground beef, particularly from dairy cattle [13, 35, 36]. Finally, subsequent to our investigation of sporadic cases of infection, multistate outbreaks of Newport-MDRAmpC infection have been associated with foods, including cheese made from unpasteurized milk and ground beef produced at a large processing plant in the Mid-Atlantic that slaughters New England dairy cattle [37, 38].

The identification and increase of Newport-MDRAmpC has important implications for human medicine. Although antimicrobials are not needed for most *Salmonella* infections, they may be life-saving in invasive infections. Ceftriaxone is commonly used for the empiric treatment of fever and sepsis in

children who are being evaluated for a source of infection, as well as for the treatment of children with invasive infections, including those due to salmonellosis. In the present study, more patients with Newport-MDRAmpC reported having bloody diarrhea, compared with persons with antimicrobial-susceptible *Salmonella* Newport infection. Although we did not demonstrate a statistically significant difference in severity of illness, as measured by hospitalization, other studies have reported that antimicrobial-resistant *Salmonella* infections are associated with increased rates of hospitalization and greater morbidity and mortality [5–7]. Further studies are needed to determine the clinical effect of Newport-MDRAmpC infections.

Prevention and control of Newport-MDRAmpC infection in humans and cattle requires an understanding of how this strain is introduced onto farms and disseminated among cattle. The management practices that promote its spread are not known but could include the use of therapeutic or prophylactic antimicrobial agents on farms, as has been suggested elsewhere [12]. Ceftiofur, an expanded-spectrum cephalosporin, is commonly used therapeutically in dairy cattle [39], whereas tetracycline and neomycin are used prophylactically in calf milk replacers [40]. Approximately 55% of dairy farms surveyed in 21 major dairy states during 2002 reported using calf milk replacer that contained medication; oxytetracycline and neomycin were the most commonly used [41]. On the farms we visited, we documented the therapeutic use of ceftiofur in ill cattle and the use of tetracycline- and neomycin-supplemented calf milk replacers. Use of these or other antimicrobial agents on farms, especially if used frequently, create a selective pressure that is likely to promote transmission and dissemination of Newport-MDRAmpC [4, 8]. On the farms we visited, we also identified tremendous movement of cattle between farms and to slaughterhouses (data not shown), which would provide ample opportunity for dissemination of Newport-MDRAmpC.

We have confirmed that Newport-MDRAmpC isolates contain genes of the bla_{CMY} family, which produce AmpC-type β-lactamases. These confer resistance to extended-spectrum cephalosporins (e.g., ceftriaxone), penicillin-inhibitor combinations (e.g., amoxicillin-clavulanate), and cephamycins (e.g., cefoxitin), the latter 2 of which distinguish the isolates from the extended-spectrum β-lactamase resistance pattern [42]. Recent molecular studies of Newport-MDRAmpC isolates indicate that the determinants of resistance to antimicrobials are present on large, transferable plasmids [24, 43]. This is of great concern, since transferable plasmids provide a molecular mechanism for the rapid emergence of MDR pathogens and may explain the rapid dissemination of Newport-MDRAmpC strains in the United States.

In Massachusetts, all the isolates with the 10 closely related PFGE patterns matching the most prevalent pattern, JJPX01.0014, were the MDRAmpC resistance phenotype. In Massachusetts, there was no overlap in PFGE patterns between the MDRAmpC isolates and the pansusceptible isolates. Since CDC usually receives only the digital images of PFGE gels and not the actual isolates, we were unable to confirm this finding among the PFGE patterns submitted to PulseNet. The analysis of data from Massachusetts suggests that these PFGE patterns can serve as a proxy for the identification of MDRAmpC strains or a variation thereof, but further studies are needed to confirm this.

Newport-MDRAmpC is both a veterinary and human public health problem about which much still needs to be learned. Preventing and controlling Newport-MDRAmpC will depend on efforts in both the public and the private sectors. Continued laboratory-based surveillance—including serotyping, antimicrobial susceptibility testing, and PFGE testing of isolates from humans, animals, and foods—is essential. These surveillance activities require increased coordination and partnership between state public health laboratories and veterinary diagnostic laboratories, as well as between state and federal agencies that address human health, animal health, food, and agriculture. Further studies are needed to evaluate risk factors for both human and animal infections. Because the overuse and misuse of antimicrobials can contribute to the dissemination of Newport-MDRAmpC, efforts that promote appropriate use of antimicrobials in both humans and animals are important. We support the FDA's effort to propose new guidelines for evaluating the safety of the use of antimicrobial agents in food animals, with regards to their microbiological effects on human bacteria [44], and recommend continued support for surveillance of the use of existing antimicrobial agents in food animals and their effect on human health. Physicians should be alerted to the rapid increase in *Salmonella* strains resistant to expanded-spectrum cephalosporins as they consider treatment options for complicated *Salmonella* infections.

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